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Variable region sequences of autoantibodies from mice with experimental systemic lupus erythematosus*

We have sequenced nine monoclonal antibodies (mAb) derived from C3H.SW mice in which experimental systemic lupus erythematosus (SLE) was induced. The hybridomas were selected for binding to DNA or to HeLa nuclear extract (NE). Three mAb were found to bind DNA, and are shown to exhibit sequence characteristics of pathogenic anti-DNA antibodies. One, mAb 2C4C2, is shown to use a heavy chain V region gene (V_H) identical to the V_H of anti-DNA mAb isolated from other lupus-prone mice, namely (NZB × NZW)F_1. The light chain V region gene (V_L) of mAb 2C4C2 is 98% homologous to the V_L of another anti-DNA mAb, also isolated from (NZB × NZW)F_1 mice. The other two anti-DNA mAb, 5G12-4 and 5G12-6, share 93% of their V_H sequences with that of mAb 2C4C2. Six mAb bound proteins of HeLa NE. Four of these six antibodies were found to use the VH124 V_H and V-L7 V_L. The nine mAb use a total of five V_H and four V_L germ-line genes, demonstrating that the autoantibodies induced in mice with experimental SLE do not originate from one B cell clone. Three of these nine V_H and V_L were identical in sequence to germ-line genes, while at least three others had somatic mutations. The latter suggests that the above autoantibodies arise in mice by both usage of existing (pre-immune) B cells, and through an antigen-driven process. Furthermore, it appears that autoantibodies found in mice with experimental SLE use genetic elements similar to those used by mAb that were isolated from mouse strains which develop lupus spontaneously.

1 Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of antibodies against various self antigens [1]. Antibodies to DNA were found to play an important role in the disease [2–4]. These antibodies have also been extensively investigated in the different murine strains which develop lupus spontaneously [2, 4]. Sequence determination has been used to study the development of autoantibodies in SLE patients and lupus-prone mice. Thus, it was shown recently that human anti-DNA antibodies can undergo somatic mutations [5, 6], and a role for antigen-driven selection of somatic mutations, progressing from IgM isotype with low affinity for DNA to IgG isotype with high affinity for DNA, was suggested [5]. Clonal expansion of B cells producing anti-DNA antibodies was also proposed to occur in lupus-prone mice. In MRL/Mp-lpr/lpr mice it was shown that B cells producing anti-DNA antibodies arise in a single mouse from a small number of clones [7–9]. A similar pattern for the development of pathogenic anti-DNA antibodies was reported in two other lupus-prone mouse strains, (SWR × NZB)F_1 [10] and (NZB × NZW)F_1 [11].

In light of the various reports indicating that the expansion of autoantibodies in SLE patients and in lupus-prone mice is oligoclonal, it is of interest to investigate the development of pathogenic autoantibodies in an inducible experimental model of murine SLE. We previously reported the induction of experimental SLE in mice which do not spontaneously develop autoimmune diseases, following immunization with either a human anti-DNA mAb bearing the 16/6 Id [12] or an anti-16/6 Id murine mAb [13]. The mice in which experimental SLE has been induced, produce high titers of autoantibodies, typical of SLE in human and in mice that develop lupus spontaneously. Furthermore, these high titers of antibodies were associated with increased erythrocyte sedimentation rates, leukopenia, proteinuria, abundance of immune complexes in the kidneys and sclerosis of the glomeruli [12].

Here we present the complete nucleotide sequences of the expressed H chain V region (VH) and L chain V region (VL) of nine autoantibodies isolated from mice with experimental SLE. Comparison of the sequences of the VH and VL shows that the autoantibodies arise in the mice in an oligoclonal manner, suggesting a role for antigen-driven stimulation. Furthermore, we found striking similarities between VH and VL sequence of mAb isolated from mice with experimental SLE, to VH and VL sequences of mAb isolated from lupus-prone mice.

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Abbreviations: **VH:** Immunoglobulin heavy chain variable region **V_H:** Heavy chain variable region gene **VL:** Immunoglobulin light chain variable region **V_L:** Light chain variable region gene **NE:** HeLa nuclear extract **FR:** Framework region **CDR:** Complementarity-determining region

Key words: Experimental systemic lupus erythematosus / Autoantibodies / Variable region sequences / Anti-DNA antibodies

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2 Materials and methods

2.1 Mice

C3H.SW and (C57BL/6 × BALB/c)F_1 female mice were obtained from the Jackson Laboratory, Bar Harbor, ME. Mice were used at the age of 8 weeks.

2.2 Human mAb

The human mAb 16/6 is an anti-DNA antibody that was derived from an SLE patient and expresses a common idiotype, the 16/6 Id [14]. This mAb is of the IgM isotype. The mAb was affinity purified on a goat anti-human IgM-Sepharose 4B column from the hybridoma supernatant.

2.3 Production of murine mAb

Experimental SLE was induced in C3H.SW mice using the IgM/κ anti-16/6 Id mAb 1A3-2, as described previously [13]. Four months following the booster injection, the mice were examined for serological and clinical manifestations typical for experimental SLE [13]. Mice with experimental SLE were injected with an additional dose of 20 μg of mAb, and after 3 days their spleens were taken for fusion as described [15]. Hybrid cells that secreted autoantibodies were cloned by limiting dilution in 96-well microtiter plates [16]. All the murine mAb used in this study were affinity purified from the hybridoma supernatants using a goat anti-mouse Ig-Sepharose 4B column.

2.4 RIA

mAb were screened and identified as described before. Briefly, flexible plastic microtiter plates (Falcon, CA) were coated with DNA or HeLa nuclear extract (NE) for 2 h. The culture supernatants were incubated for 4 h, followed by incubation for 18 h with ^{125}I-labeled goat anti-mouse Ig (1×10^5 CPM/well). Radioactivity was measured using a γ counter.

2.5 Immunobloting

Immunological detection of gel-separated nuclear proteins by the culture supernatants was done as described previously [17]. Briefly, HeLa NE proteins were run on an 8.75% polyacrylamide gel in the presence of SDS. The gel separated proteins were transferred to a nitrocellulose sheet, and then incubated for 1–2 h with the culture supernatants, diluted 1:200. The strips were extensively washed, and incubated for 1 h with ^{125}I-labeled goat anti-mouse Ig (4×10^5 cpm/ml). After washing, the filters were dried and autoradiographed.

2.6 RNA preparation

Total RNA was isolated from hybridoma cells as described [18]. Briefly, 10^8 cells were incubated in high salt mixture, in the presence of 0.5% NP40 (Sigma, St. Louis, MO). Thereafter the nuclei were precipitated, and the supernatant, which contained total RNA, was mixed with an equal volume of 7 M urea Tris buffer. Subsequently, the proteins were removed by phenol/chloroform extraction. Following ethanol precipitation, about 750 μg total RNA was recovered from 10^8 cells.

2.7 Cloning and sequencing of antibody V regions

Total RNA (20–30 μg) from all mAb was incubated with isotype-specific primers, and first strand cDNA was synthesized using reverse transcriptase (USB, Cleveland, OH). The cDNA was then subjected to PCR amplification, using Taq polymerase (Promega, Madison, WI) with forward primers located in the constant region or in the J region, and backward primers located in the V region, close to the leader sequences [19]. PCR products were cloned in SmaI site of M13 and were sequenced using Sequenase 2.0 kit (USB).

3 Results

3.1 Induction of experimental SLE and generation of mAb

Experimental SLE was induced in C3H.SW female mice by immunization with an anti-16/6 Id mAb (mAb 1A3-2, [13]). Four months later, two mice were killed, and their spleen cells were fused with IdX-63 myeloma B cells. The characteristics of nine of the hybridoma clones obtained, are summarized in Table 1. As can be seen, three mAb were found to bind DNA. Two of these also bear the 16/6 Id, as detected using rabbit anti-16/6 Id sera (Waisman et al., manuscript submitted). The 2C4C2 mAb bound cardiolipin in addition to DNA [20]. The other six mAb bound NE proteins, but did not bind DNA, and did not express the 16/6 Id. One of the latter mAb, 2D12, was found previously to bind the La protein (SS-B) [17, 21]. mAb 3B12-1 was found by western blot analysis to bind two bands of 110 and 90 kDa of HeLa NE (data not shown). The other four mAb bind a splicing factor of 88 kDa (data not shown), termed splicing factor 53/4 (SF53/4), which has previously been described by Ast et al. [22].

3.2 Variable region sequences of the mAb

3.2.1 H chain sequences

Table 1 summarizes the genetic elements of the nine mAb described in the present report. As can be seen in the table, the VH of the nine mAb arise from five different germ-line genes, and the VL from four different germ-line genes.

Fig. 1 shows the nucleotide (1a) and the putative amino acid (1b) sequences of the VH regions of the autoantibodies isolated from mice with experimental SLE, in comparison to the sequences of previously described mAb. As seen in Fig. 1, the sequence of the V_H-encoded region of the anti-DNA anti-cardiolipin mAb 2C4C2 is identical to that of another anti-DNA mAb, BW16. The latter was isolated from (NZB × NZW)F_1 mice [23].

Table 1. Summary of sequence characteristics of monoclonal autoantibodies isolated from mice with experimental SLE

Designation	Isotype	Specificity	V_κ group[a]	Similar V_κ gene segment	J_κ	V_H Family[b]	Similar V_H gene segment	D_H	J_H	CDR3[c] length
2C4C2	IgM;κ	Anti-cardiolipin, anti-DNA	2	NZB × W Anti-DNA [23][d]	1	J558	NZB × W Anti-DNA [23]	NA[e]	3	21
5G12-4	IgG2a;κ	Anti-DNA, 16/6 Id$^+$	12	CB.20 NF 12B11S [30]	2	J558	C57BL/6 germ line [26]	NA[e]	4	30
5G12-6	IgG2a;κ	Anti-DNA, 16/6 Id$^+$	ND	ND	ND	J558	C57BL/6 germ line [26]	NA[e]	4	90
3B12-1	IgM;κ	Anti-RNP	23	BALB/c germ line [31]	2	J558	BALB/c germ line [37]	SP2.3	3	24
3B12-2	IgM;κ	Anti-88 kDa splicing factor	23	BALB/c germ line [31]	2	J558	BALB/c germ line [37]	SP2.3	3	24
3B12-3	IgM;κ	Anti-88 kDa splicing factor	9	BALB/c germ line [32]	4	J558	BALB/c germ line [37]	SP2.3	3	24
2C5-3	IgM;κ	Anti-88 kDa splicing factor	23	BALB/c germ line [31]	2	J558	BALB/c germ line [37]	SP2.3	3	24
3A2-22	IgM;κ	Anti-88 kDa splicing factor	9	BALB/c germ line [32]	2	Q52	P8A[e]	SP2.2	4	51
2D12	IgM;κ	Anti-La (SS-B)	23	BALB/c germ line [31]	2	36–60	BALB/c GAT [28]	NA[f]	3	15

a) V_κ group number designations are according to Potter et al. [44].
b) V_H family classification is according to Dildrop [45].
c) CDR3 length is defined as the number of base pairs between the last codon of FR3 (the invariant Arg residue) and the invariant Trp residue encoded by each of the J_H genes.
d) In brackets, the original article corresponding the reference sequence.
e) Flaswinkel & Reth, mouse rearranged VH gene P8AQ52, EMBL entry MMQ52P8A.
f) Not assignable to any known group or gene sequence.

In contrast to the similarity of the V_H the third complementarity-determining region (CDR3) of mAb 2C4C2 shows very little homology in sequence to that of mAb BW16 (Fig. 1). It should be noted that the CDR3 of 2C4C2 contains an arginine residue encoded in the D segment (Fig. 1b), which was demonstrated to play an important role in binding to negatively charged DNA [24, 25].

Two other anti-DNA mAb isolated from the mice with experimental SLE, 5G12-4 and 5G12-6, were found to bear the 16/6 Id. Their VH sequences are compared in Fig. 1 to that of mAb 22.11 which was found to bind the NP hapten [26], and its V gene sequence was reported to be coded by a non-mutated germ-line gene [27]. As can be seen in Fig. 1b, the V_H of mAb 5G12-4 and 5G12-6 differ from the V_H of the anti-NP mAb 22.11 by six nucleotides, resulting in four amino acid replacements (Table 2). As seen in Fig. 1, mAb 5G12-4 and 5G12-6 have the same VDJ sequences.

Sequence of the VH of the six anti-NE mAb revealed four which are coded by the same germ-line gene. As can be seen in Fig. 1a, mAb 3B12-1, 3B12-2, 3B12-3 and 2C5-3, were found to be almost identical to the germ-line gene VH124 of the J558 family. All four mAb use the same D and J segments, implying that they arise from the same pre-B cell.

As depicted in Fig. 1, the VH sequence of mAb 3A2-22 differs in its V_H from the sequence of the V_H of the P8A gene of the Q52 family (Flaswinkel & Reth, mouse rearranged VH gene P8AQ52, EMBL entry MMQ52P8A) by only one nuclotide, located in the CDR2. This suggests that the V_H of mAb 3A2-22 is most likely derived from the P8A germ-line gene.

The VH sequence of mAb 2D12, which binds the La protein [21], is also shown in Fig. 1. As can be seen in the Fig., its V_H encoded region is 98 % homologous to that of mAb HP 22 of the V_H 36–60 family, which was isolated from BALB/c mice [28]. Although these two mAb use different D segments, there are only six nucleotide substitutions in the V_H, resulting in six amino acid replacements (Table 3).

Table 2. Amino acid differences between the V_H of mAb 5G12-4, 5G12-6 and 22.11

	Amino acid position	22.11	5G12-4,6
CDR1	34	Val	Met
	35	Asn	Gln
CDR2	59	Asn	Tyr
	65	Ser	Ala

Table 3. Amino acid differences between the V_H of mAb 2D12 and HP 22

	Amino acid position	HP 22	2D12
CDR1	31	Arg	Ser
	34	Asn	Tyr
FR2	39	Arg	Gln
CDR2	52	Asn	Ser
FR3	69	Val	Ile
	82	Met	Leu

Table 4. Amino acid differences between the V_L of mAb 2C4C2 and BW14

	Amino acid position	BW14	2C4C2
FR1	19	Ala	Val
CDR1	26	Asn	Ser
	30	Glu	Lys
FR3	60	Asn	Asp
	76	Ser	Ile
	80	Ala	Val
J segment		Jκ2	Jκ4

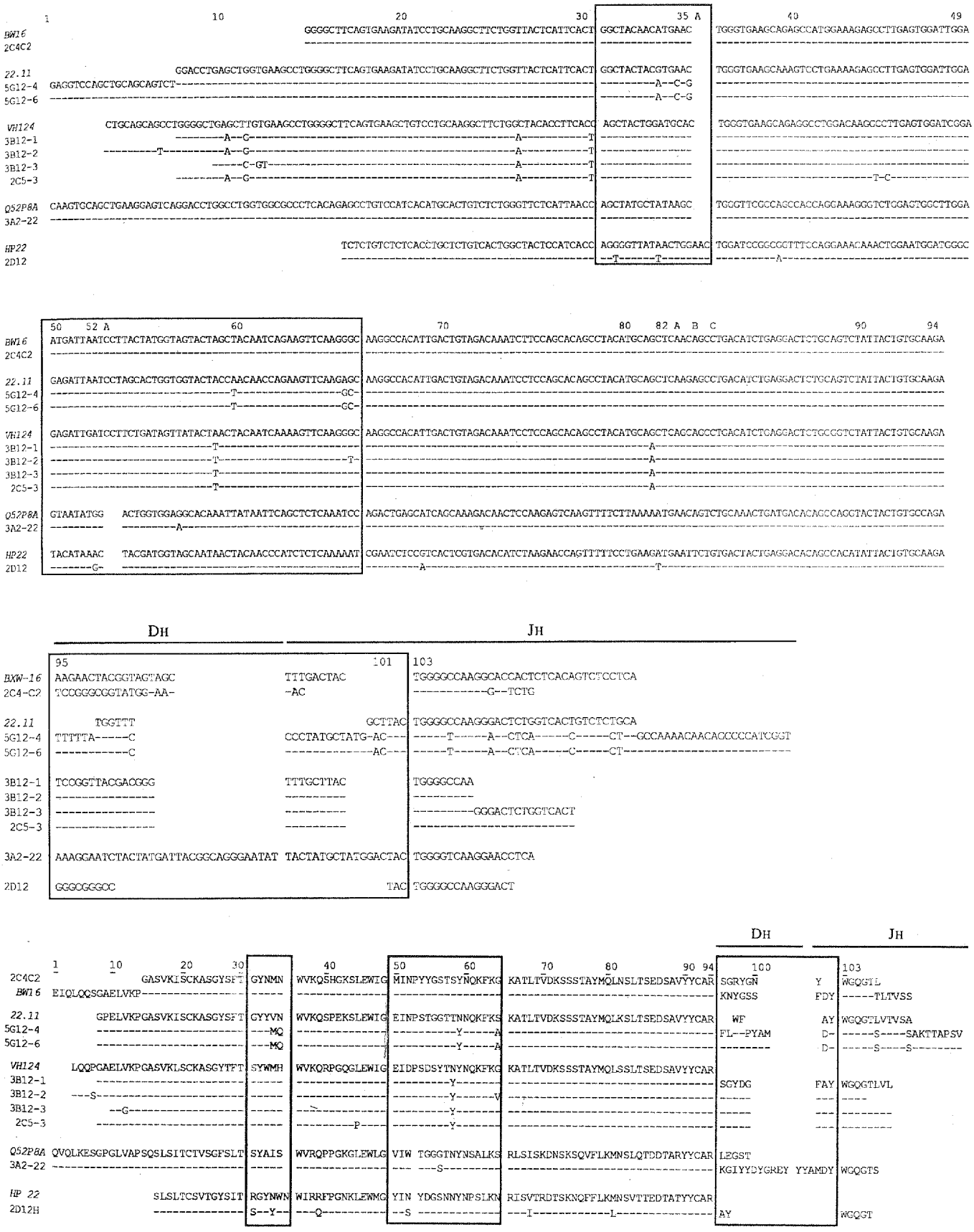

Figure 1. Nucleotide (A) and deduced amino acid (B) sequences for VH. The sequences are grouped according to the V_H gene, from which each VH was derived. The VH sequences are compared to reference sequences (in italics). Dashes indicate identity with the reference sequences; blanks indicate either that the VH does not have an amino acid at that position or sequence was not obtained for that region. CDR regions are boxed. The J_H and D_H regions are indicated. Amino acid numbers are according to Kabat et al. [43]. The nucleotide sequences have been submitted to the EMBL/GenBank Sequences Libraries.

Three differences are located within the CDR, and three in the framework regions (FR).

3.2.2 L chain sequences

The sequence of the VL of mAb 2C4C2, in comparison to an anti-DNA mAb, BW14, isolated from (NZB × NZW)F_1 mice [23], is shown in Fig. 2. As can be seen in the figure, 97% homology was found between the V_L of mAb 2C4C2 and BW14, which was previously found to be identical to the V_L of an anti-histone mAb [29]. As summarized in Table 4, mAb 2C4C2 and BW14 differ by six amino acid residues within their V_L, two in the CDR1, and four in the FR. This suggests that the VL of mAbs 2C4C2 and BW14 are encoded by similar, though different germ-line genes.

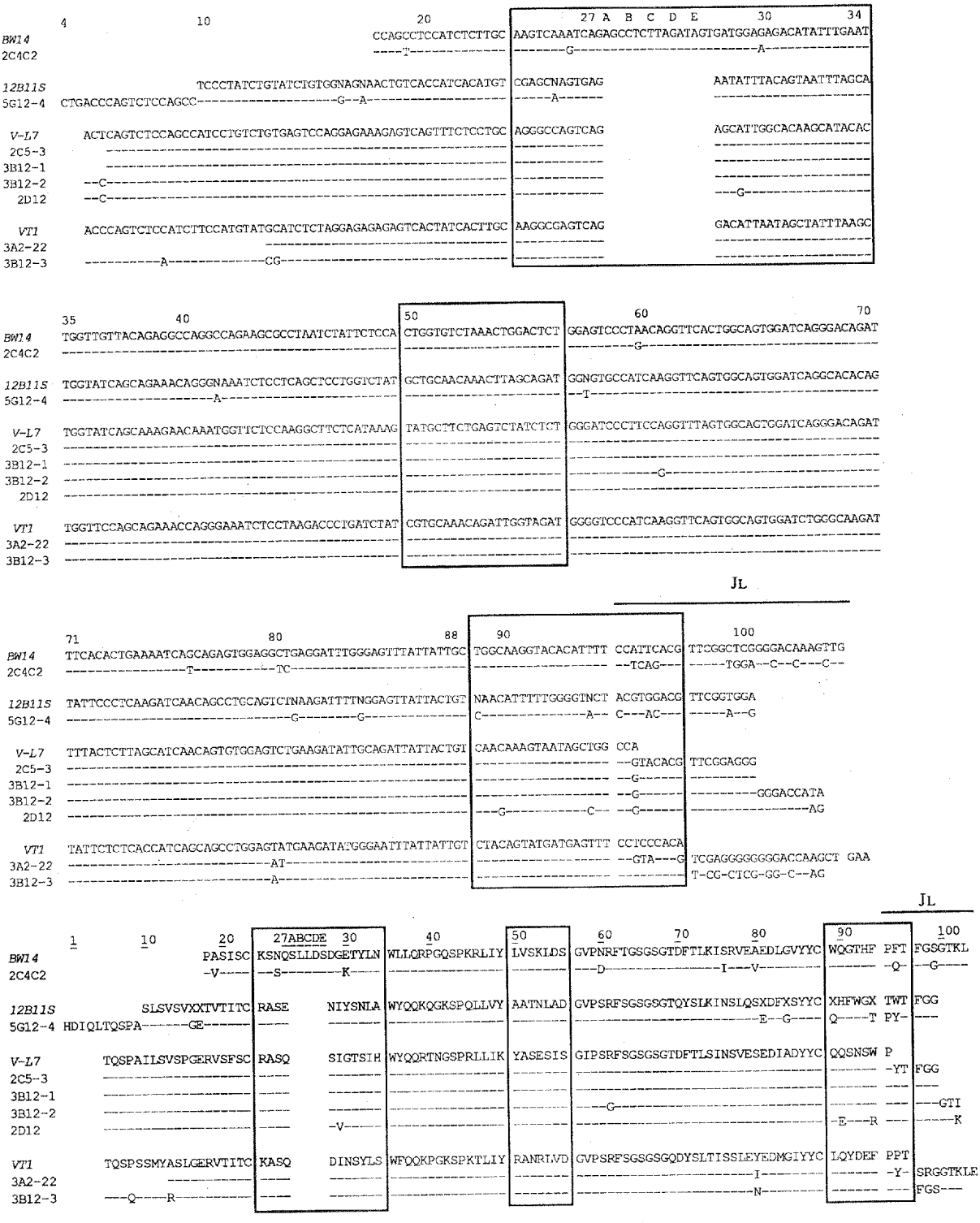

Figure 2. Nucleotide (A) and deduced amino acid (B) sequences for VL. The organization of the sequences is as in Fig. 1. Amino acid numbers are according to Kabat et al. [43]. The nucleotide sequences have been submitted to the EMBL/GenBank Sequences Libraries.

As can be seen in Fig. 2, the V_L sequence of mAb 5G12-4 is identical to that of mAb 12B11S [30]. The latter was isolated from Ly-1-positive B cells, but not tested for its specificity.

Four mAb were found to be coded by the same V_L germ-line gene, V-L7 [31]. As demonstrated in Fig. 2, mAb 2C5-3 and 3B12-1 show complete sequence homology to V-L7 in their V_L-encoded region. mAb 3B12-2 was found to have two nucleotide substitutions in comparison to V-L7, resulting in glycine to arginine substitution at position 61 of FR3. mAb 2D12 differs in its V_L-encoded region from V-L7 by four nucleotides, resulting in three amino acid substitutions in the CDR 1 and 3.

The sequence of the VL of two additional mAb, 3A2-22 and 3B12-3, is shown in Fig. 2. As seen in the figure, these mAb were found to be highly homologous to the VT1 germ-line gene, previously shown to code for the VL of the tumor myeloma T [32]. As depicted in Fig. 2, mAb 3A2-22 differs from VT1 by two nucleotides, leading to one amino acid substitution, at position 80 in the FR3. mAb 3B12-3 differs from VT1 by three amino acids, two of which are in the FR1. In addition, mAb 3A2-22, 3B12-3 and VT1 were each found to utilize a different J_L segment.

3.3 Sequence comparison

Seven mAb described in Fig. 1 are coded by V_H of the J558 family, and show more than 85 % homology with each other (see Table 5). In contrast, the V_H of the other two mAb described here, 3A2-22 and 2D12 (Fig. 1), have completely different sequences, that are derived from different V_H families. As seen in Table 5, the VL regions were found to be coded by four different germ line genes, exhibiting 60–70 % homology. Some mAb, *e.g.* 2D12 and 3B12-1, were almost identical in their VL, but showed low homology in their VH. In constrast, mAb 3B12-1 and 3B12-3 shared 97 % homology in their VH, but only 63 % homology in their VL (Table 5).

4 Discussion

In the present study we report the sequencing of variable regions of nine mAb isolated from C3H.SW mice with experimental SLE. Sequencing of the VH of the mAb revealed that they are coded by five different germ-line genes, while their VL are coded by four different germ line genes. In addition, we found striking similarities between the V_H and V_L sequences of our anti-DNA mAb and anti-DNA mAb isolated from lupus-prone mice.

SLE is a systemic autoimmune disease characterized by the formation of antibodies against self antigens, such as DNA, RNP, Sm, Ro and La [1, 33]. The etiology of SLE is unknown, and understanding the mechanism by which these anti-self antibodies arise might provide insight to this problem. We sequenced the V regions of nine autoantibodies that bind either DNA or NE, isolated from C3H.SW mice with experimental SLE. We analyzed mAb with different specificity, in an attempt to determine the connections between the different autoantibodies.

Table 5. Nucleotide and amino acid homology between mAb isolated from mice with experimental SLE[a,b]

Nucleotide Homology (%)

Amino Acids Homology (%)

	5G12-4,6	2C4C2	3B12-1	3B12-2	3B12-3	2C5-3	3A2-22	2D12
5G12-4,6		H 88 (92) L 57 (56)	H 83 (86) L 64 (63)	H 85 (86) L 64 (63)	H 82 (86) L 71 (72)	H 82 (85) L 64 (63)	H 61 (57) L 72 (72)	H 55 (57) L 64 (62)
2C4C2	H 80 (85) L 40 (40)		H 81 (87) L 58 (57)	H 86 (86) L 57 (57)	H 84 (87) L 61 (61)	H 81 (86) L 58 (57)	H 55 (55) L 64 (63)	H 57 (59) L 58 (56)
3B12-1	H 76 (78) L 56 (54)	H 76 (78) L 52 (50)		H 99 (99) L 99 (99)	H 97 (95) L 63 (63)	H 99 (99) L100 (100)	H 53 (54) L 64 (63)	H 57 (59) L 99 (99)
3B12-2	H 80 (79) L 56 (54)	H 77 (77) L 48 (46)	H 98 (98) L 99 (99)		H 96 (95) L 63 (63)	H 99 (98) L 99 (99)	H 53 (53) L 63 (63)	H 53 (53) L 97 (99)
3B12-3	H 76 (77) L 60 (61)	H 77 (80) L 53 (51)	H 97 (97) L 48 (47)	H 96 (96) L 49 (47)		H 96 (94) L 63 (63)	H 59 (57) L 93 (97)	H 53 (53) L 63 (63)
2C5-3	H 76 (77) L 56 (54)	H 76 (78) L 52 (50)	H 99 (99) L100 (100)	H 98 (97) L 90 (99)	H 97 (97) L 48 (47)		H 54 (55) L 64 (63)	H 58 (54) L 99 (99)
3A2-22	H 51 (43) L 61 (62)	H 46 (45) L 53 (52)	H 44 (42) L 49 (48)	H 41 (41) L 49 (46)	H 45 (43) L 91 (95)	H 43 (41) L 49 (48)		H 63 (64) L 64 (62)
2D12	H 41 (38) L 56 (54)	H 45 (47) L 49 (46)	H 43 (42) L 97 (97)	H 40 (40) L 95 (95)	H 44 (43) L 49 (46)	H 43 (41) L 97 (97)	H 60 (60) L 48 (45)	

a) Percent homology is calculated from the number of similar residues per length of comparison, using the sequence analysis package, GCG7 [46].

b) Left number shows percent homology of whole chains; right number (in parentheses) shows percent homology of V gene segment.

The anti-DNA mAb 2C4C2 was found to be identical in its V_H sequence to an anti-DNA mAb (BW16) isolated from (NZB × NZW)F_1 mice (Fig. 1). Both mAb 2C4C2 and BW16 were found to be of the IgM isotype. Since these two mAb were isolated from different mouse strains, we can assume that their sequences are derived from an unmutated germ-line gene of the J558 family. mAb 2C4C2 is identical in its V_H to two anti-DNA mAb, BW16 [23] and 17s.128 [11], isolated independently from (NZB × NZW)F1 mice (Table 6). Four more mAb which were isolated from lupus-prone mice were found to share high homology with mAb 2C4C2 in the V_H [11, 34, 35]. All those mAb differ in their V_H by one to three amino acids from mAb 2C4C2 V_H (Table 6). Since they all were found to bind DNA (except MRB6 which binds a histone protein), it is very likely that they are encoded by a pathogenic V gene. This suggestion is strengthened by the fact that these hybridomas were isolated from different murine models of lupus. The expression of this V heavy gene, designated BW16, may therefore most likely indicate the appearance of autoimmune processes in the mouse.

The V_L-encoded region of mAb 2C4C2 was found to be homologous in 97 % to the V_L of another anti-DNA mAb, BW14 (see Fig. 2). Since these two mAb differ by six amino acids (Table 2), it is not clear whether they derive from the same germ-line gene or two very similar germ-line genes.

The VH of hybridomas 5G12-4 and 5G12-6 is most likely encoded by the same germ-line gene as the anti-NP mAb 22.11 [26]. The location of six nucleotide differences in the V_H-encoded regions of hybridomas 5G12-4, 5G12-6 and 22.11 in the CDR1 and CDR2 (Table 3), suggests that they might be generated by somatic mutations. The fact that mAb 5G12-4 and 5G12-6 are both of the IgG2a isotype, in contrast to mAb 2.11 which is of the IgM isotype [26], supports the likelihood that differences between them are a result of somatic mutations.

Recently, O'Keefe et al. [36] showed striking sequence similarity between the V_H-encoded regions of a group of anti-DNA hybridomas, and that of anti-NP hybridomas isolated in the same fusion as mAb 22.11. They suggested that the lack of DNA binding in the anti-NP antibodies is due to different D_H and J_H segment usage. In agreement, we found that the V_H of four hybridomas described in the present study, 3B12-1, 3B12-2, 3B12-3 and 2C5-3, are very similar in sequence to the V_H germ-line gene VH124, found to code for anti-NP mAb, and differ in their D_H and J_H segments usage [37].

The CDR3 of the H chain of hybridomas 5G12-4 and 5G12-6 could not be assigned to any known murine D_H segment [38]. However, comparison of this area to all known sequences in the data bank surprisingly reveals resemblance to the sequence of a human D_H segment [39]. Since mAb 5G12-4 and 5G12-6 bear the 16/6 Id, and since their V_H- and V_L-encoded regions were found to code for other mAb (Figs. 1 and 2), we propose that this idiotype is coded only or mainly by the CDR3 of the VH.

Three anti-NE mAb were found to be coded by the same V_H, VH124 (Fig. 1), and V_L, VL-7 (Fig. 2). It is note worthy, that mAb 3B12-3, coded by VH124 as well, and bear the same VDJ sequence as mAb 3B12-1, 3B12-2 and 2C5-3, was found to be coded by a different V_L gene, VT1 (Fig. 2). The fact that hybridomas 3B12-1,2,3 and 2C5-3 share the same VDJ sequence, suggests that they arise from the same pre-B cell. mAb 2D12, which is coded by a germ-line gene from the 36–60 V_H family, also uses the V-L7 for its L chain, as mAb 3B12-1, 3B12-2 and 2C5-3 (Fig. 2), suggesting an important role for V-L7 in the autoimmune process. It thus appears that anti-NE antibodies isolated from mice with experimental SLE primarily use the H chain germ-line gene VH124 and the L chain-germ line gene V-L7.

The VH of two mAb, 3A2-22 and 3B12-3, are coded by the same germ-line gene VT1 (Fig. 2). The V_H of these mAb differ in only one to three nucleotides from the germ-line gene. These differences probably do not affect the antibody specificity, since both mAb 3A2-22 and 3B12-2, which differ in these position, bind the same antigen (SF53/4).

The fact that the hybridomas isolated from mice with experimental SLE utilize five V_H and four V_L germ-line genes (Table 1), indicate that they are not a product of monoclonal B cell activation. On the other hand, the enhanced use of VH124 (Fig. 1) and VL-7 (Fig. 2) by the anti-NE mAb contradicts polyclonal B cell activation in the induction of experimental SLE. Thus, we propose that autoantibodies involved in experimental SLE arise from a limited number of clones. This is consistent with other reports that suggest that anti-DNA antibodies from SLE

Table 6. Amino acid differences between the V_H of mAbs 2C4C2 and similar anti-DNA mAb

	Amino acid position	2C4C2	BW16	165.14	17s.128	C72	H45-5	MRB6
FR1	13	Lys	Lys	Lys	Lys	Lys	Asn	Asn
CDR1	31	Gly	Gly	Gly	Gly	Asp	Gly	Gly
	33	Asn	Asn	Asn	Asn	Lys	Asn	Asn
FR2	39	Glu	Glu	Glu	Leu	Glu	Glu	Glu
CDR2	55	Gly	Gly	Gly	Ile	Gly	Gly	Gly
	56	Ser	Ser	Ser	Thr	Ser	Ser	Ser
FR3	75	Ser	Ser	Ser	Ser	Ser	Ser	Thr
	88	Ala	Ala	Ala	Ala	Thr	Ala	Ala
Reference			[23]	[11]	[11]	[35]	[34]	a)

a) Novick et al., Polyreactive IgM antibodies generated from autoimmune mice and selected for histone-binding activity, EMBL entry MMVHMRB6.

patients or lupus-prone mice are generated by an oligoclonal manner [7–9, 11, 23, 40–42].

The sequence of the variable region encoding regions of the nine mAb isolated from mice with experimental SLE should enable the synthesis of peptides to be used in attempts to induce experimental SLE, in order to elucidate the pathogenic epitopes in autoimmune diseases.

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